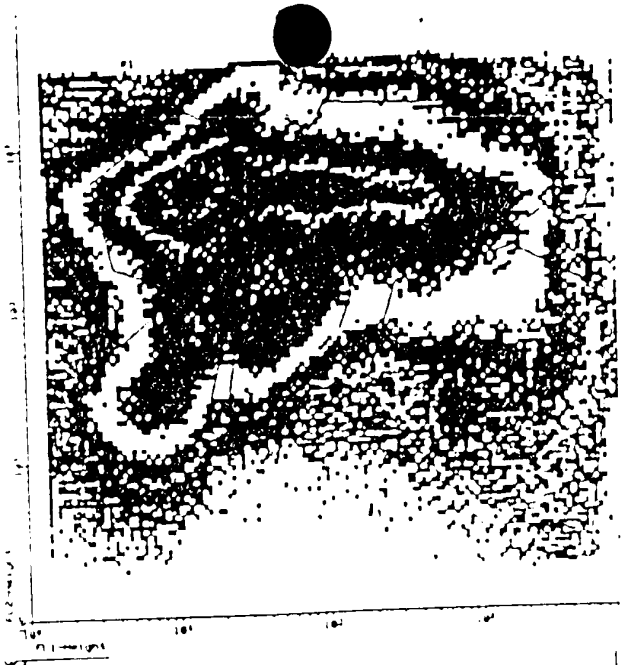
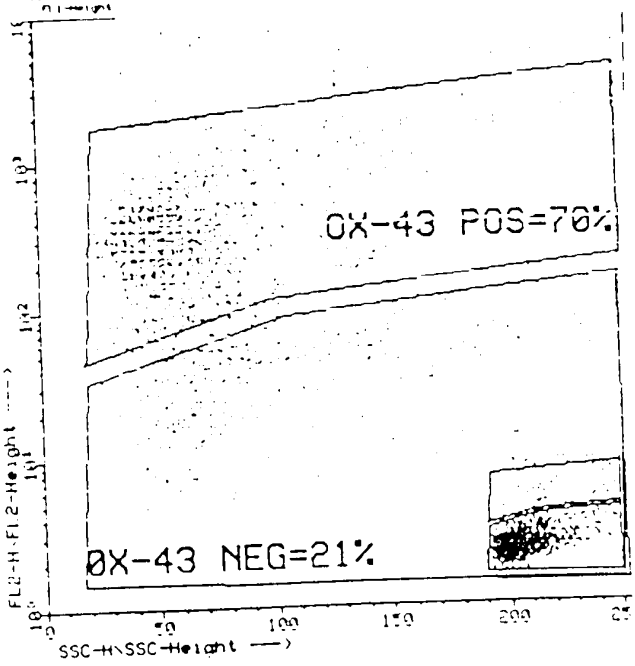
Figure 1

A



B



C

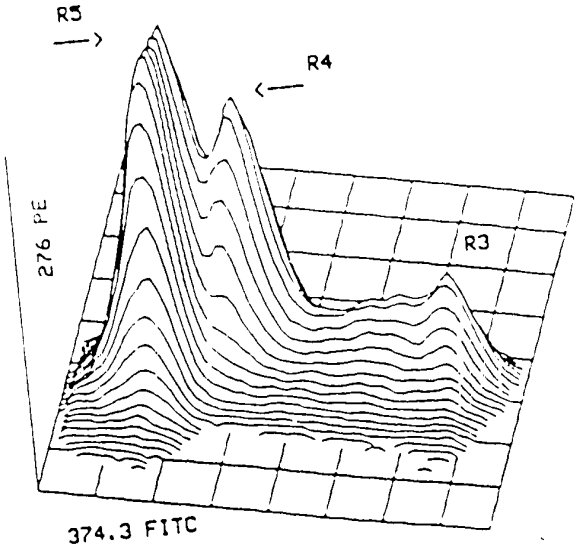


Figure 2

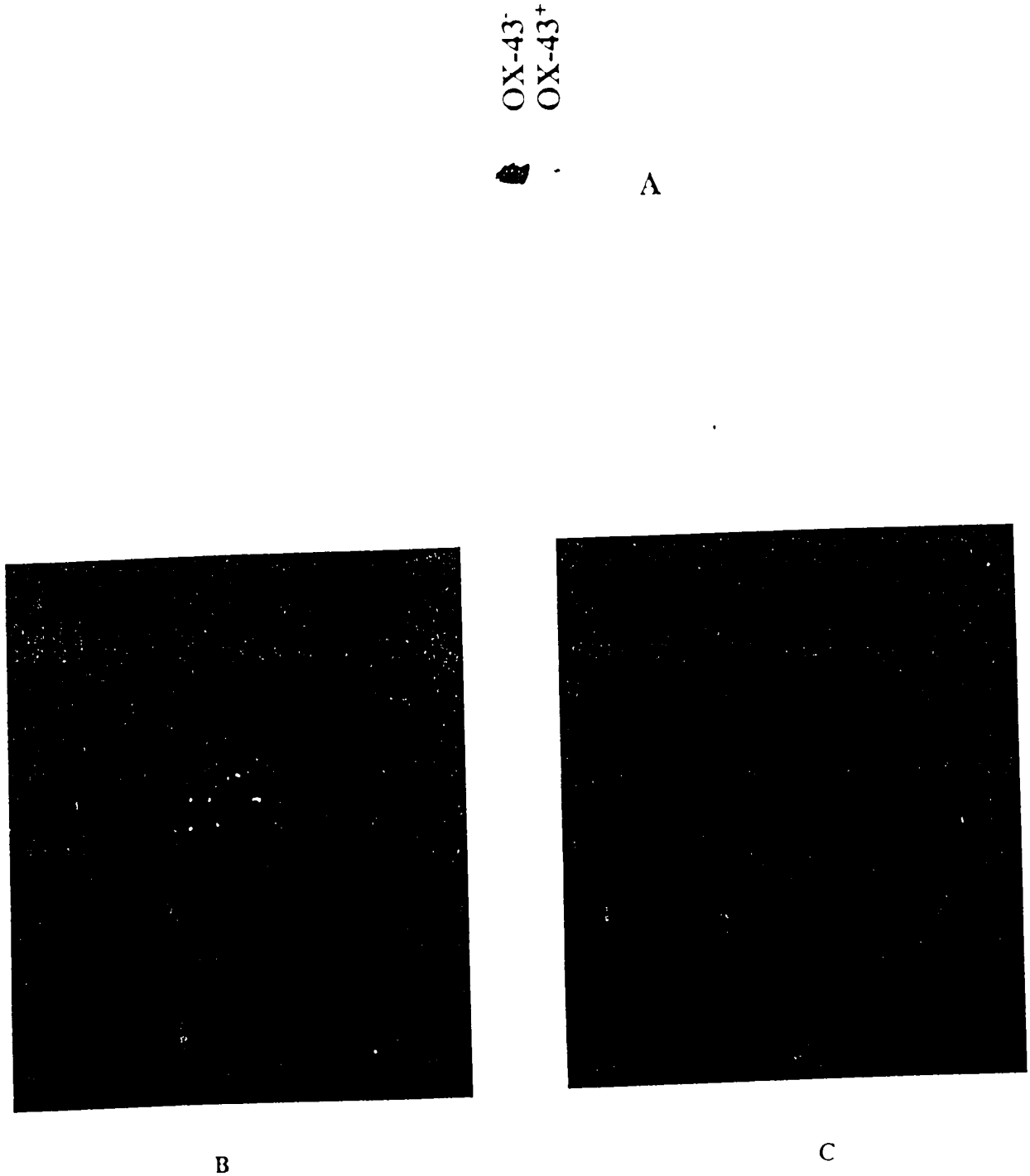


Figure 3

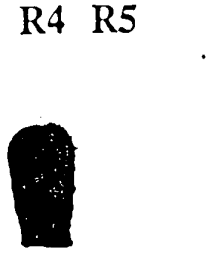


Figure 4

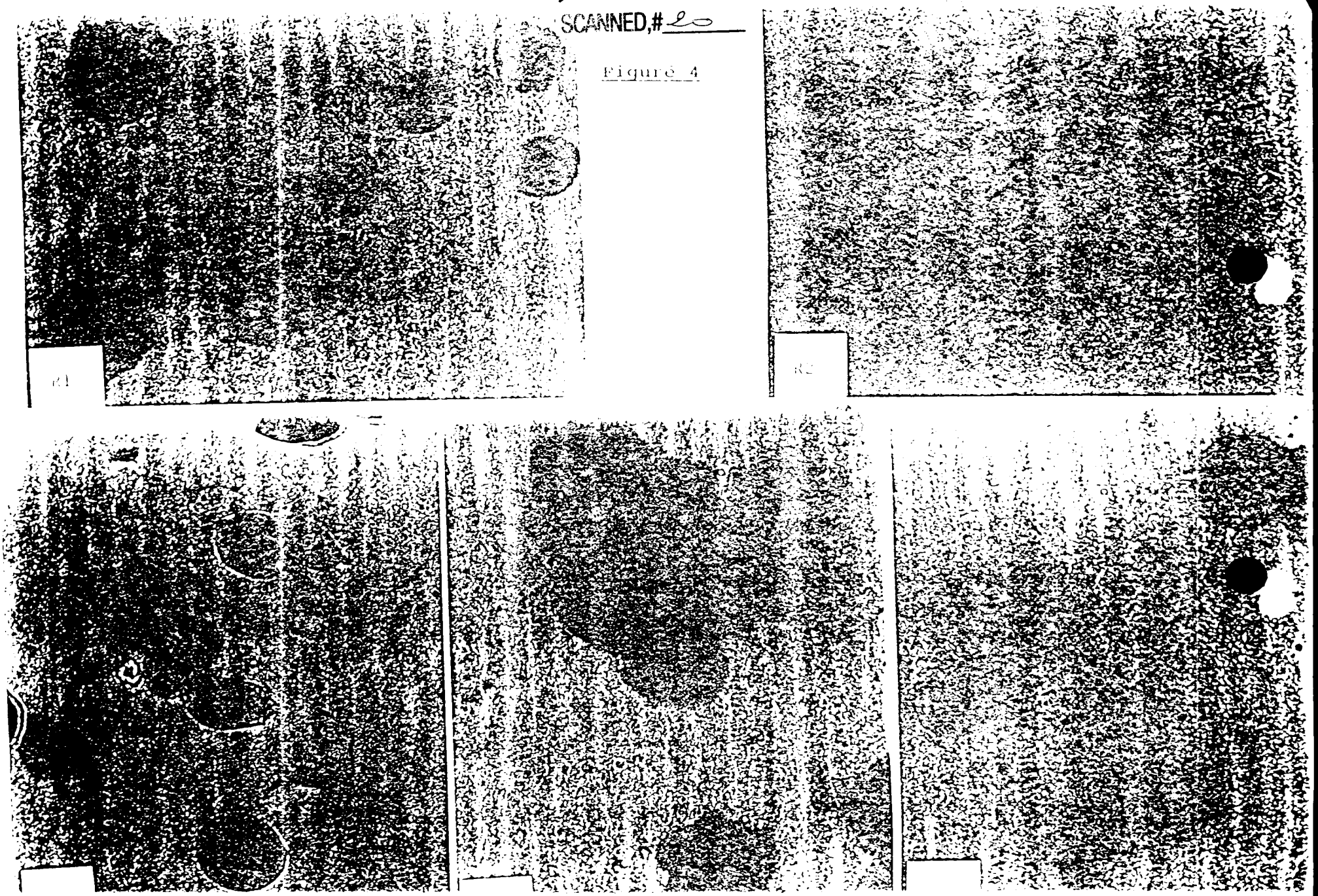


Figure 5

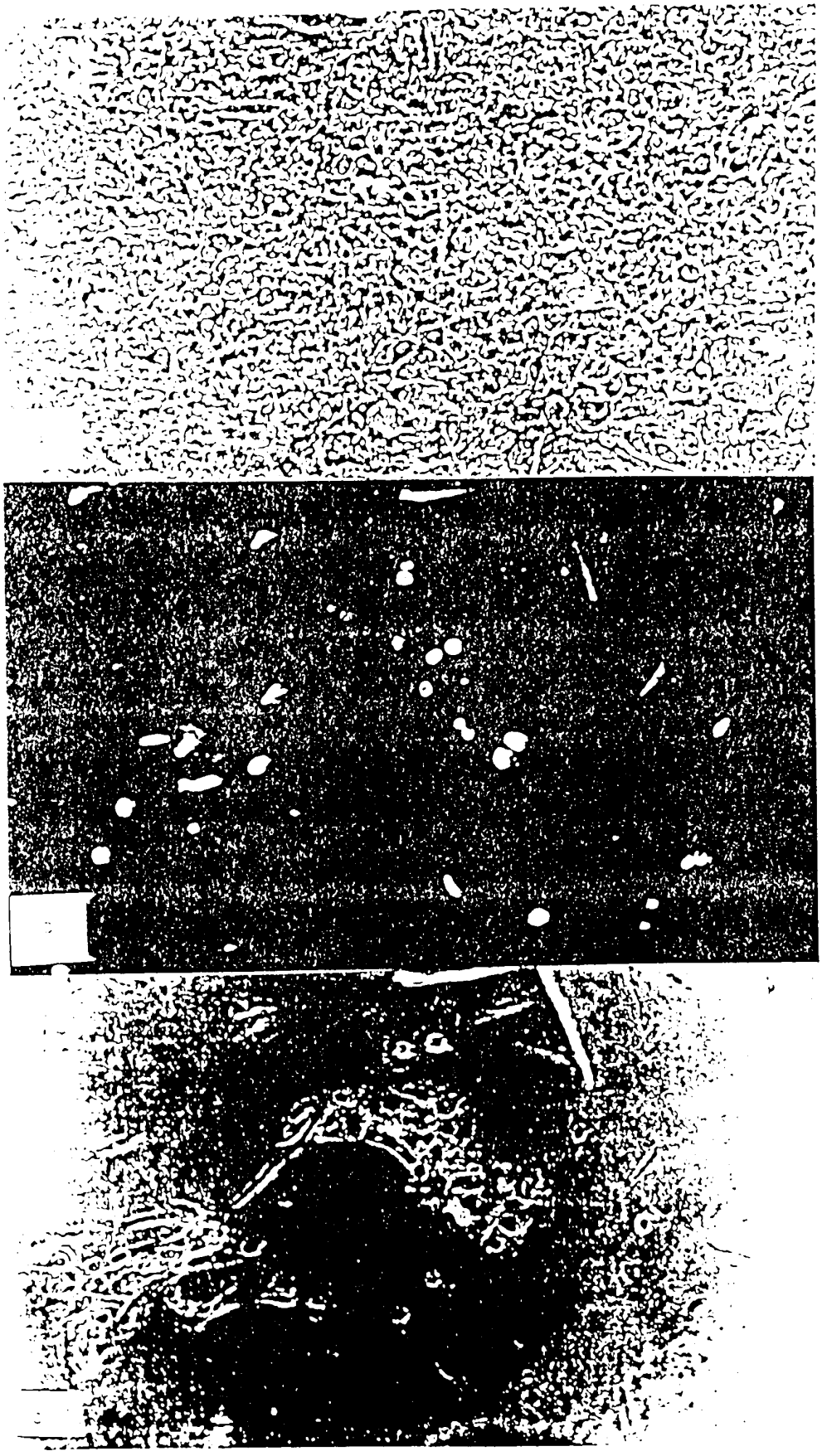


Figure 6

Flow Diagram of Hepatoblast Enrichment

Livers (8-9 mgs)

↓ Dispersion with EGTA and then collagenase

Single Cell Suspension Preparation: Collagenase, EGTA, 4°C

↓ 10^7 cells/8 mgs liver

↓ 3.2 ± 1.3% are ALB^+

↓ 2.5 ± 0.7% are AFP^+

↓ 87.9 ± 2.5% are $OX43/44^+$

Panning

Red Blood Cell Panning (2X)

↓ 29 ± 5% of cells remain

↓ 9.5 ± 1.2% are ALB^+

↓ 9.8 ± 0.9% are AFP^+

↓ 80.4 ± 3.9% are $OX43/OX44^+$

OX-43/OX-44 Panning (myeloid and endothelial cells)

↓ 16 ± 4% of cells remain

↓ 14.8 ± 3.6% are ALB^+

↓ 14.9 ± 2.5% are AFP^+

↓ 69 ± 10% are $OX43/OX44^+$

Fluorescence Activated Cell Sorting

Negatively Sort for Contaminant Cell Populations:

OX-43 (CD)/OX-44 (CD37)$^+$ Cells = precursors and mature forms of hemopoietic cells (myeloid, erythroid) and endothelial cells

Of remaining cells (OX-43$^-$ + OX-44$^-$ cells), sort for cells varying in OC.3 expression and granularity:

OX-43/(CD)/OX-44 (CD37)$^-$ Cells = mostly hepatic precursors, some residual hemopoietic cell contaminants, stromal cells

OC.3$^+$, granular cells = committed bile duct precursors (AFP^+, ALB^-)

OC.3$^-$, granular cells = committed hepatocyte precursors (AFP^+, ALB^{+++})

OC.3$^+$ agranular cells = early hepatoblasts (AFP^{+++}, albumin$^+$ and CK 19$^-$)

SCANNED,# 20

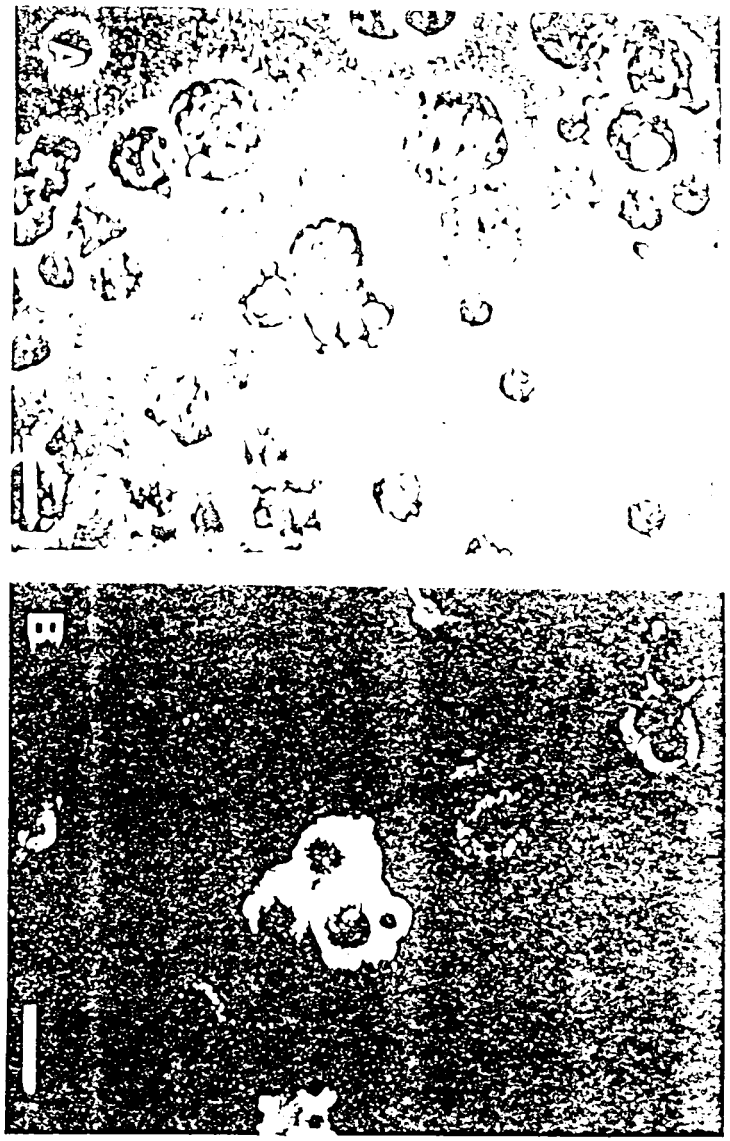


Figure 8

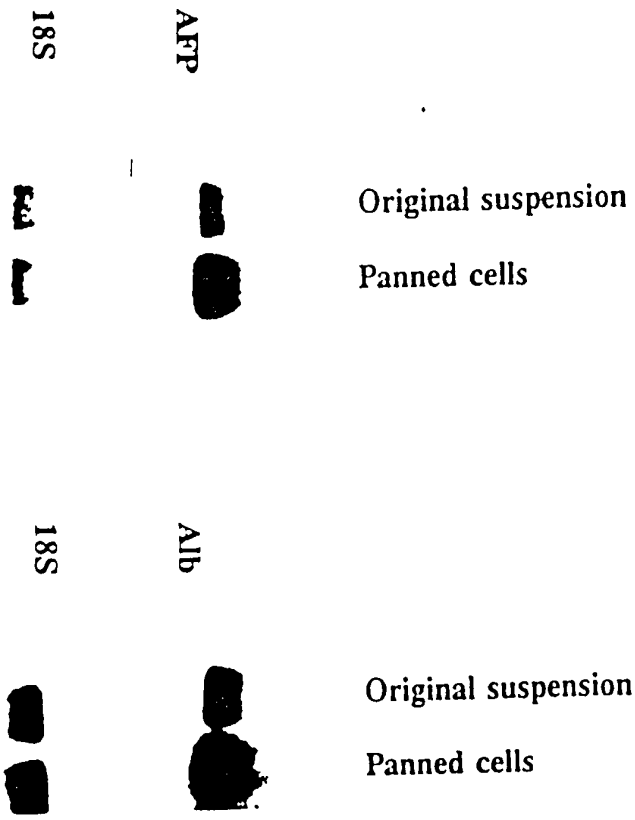
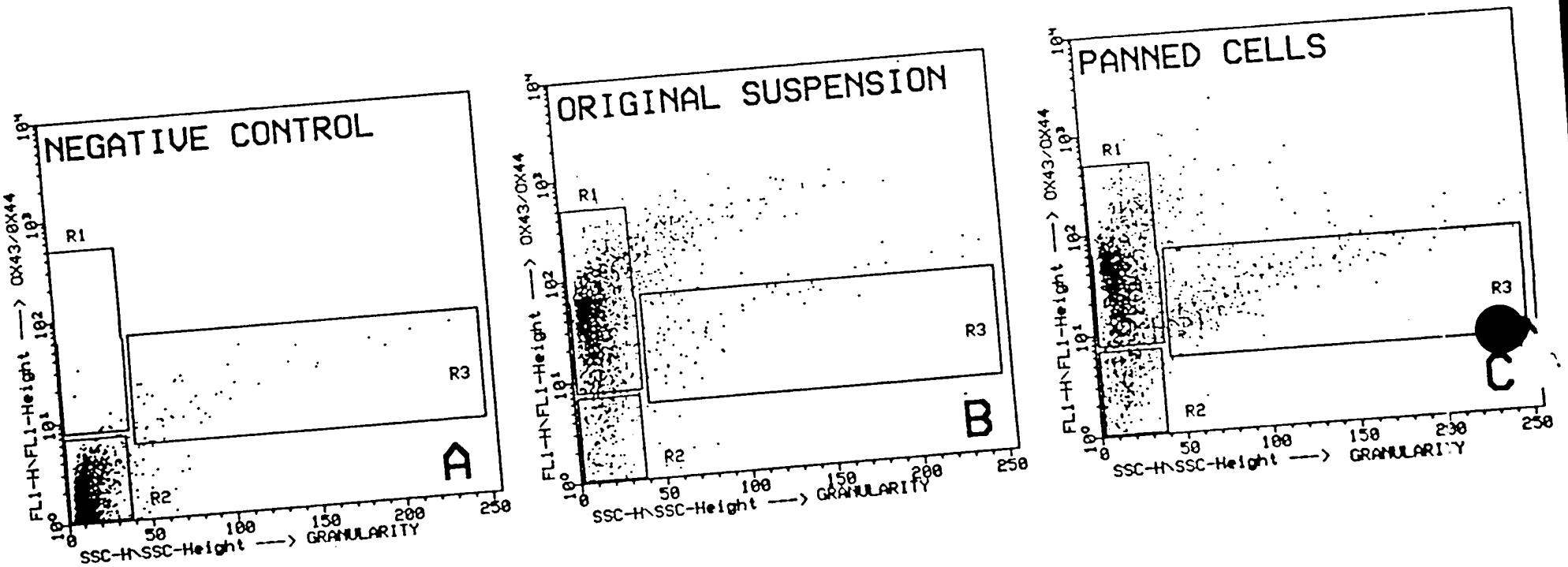


Figure 9



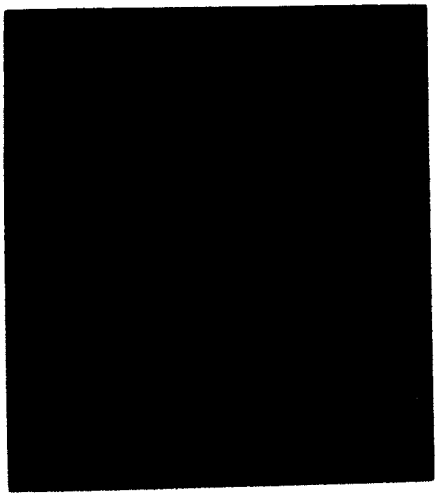


FIG. 2C

FIG. 2A

FIG. 2B

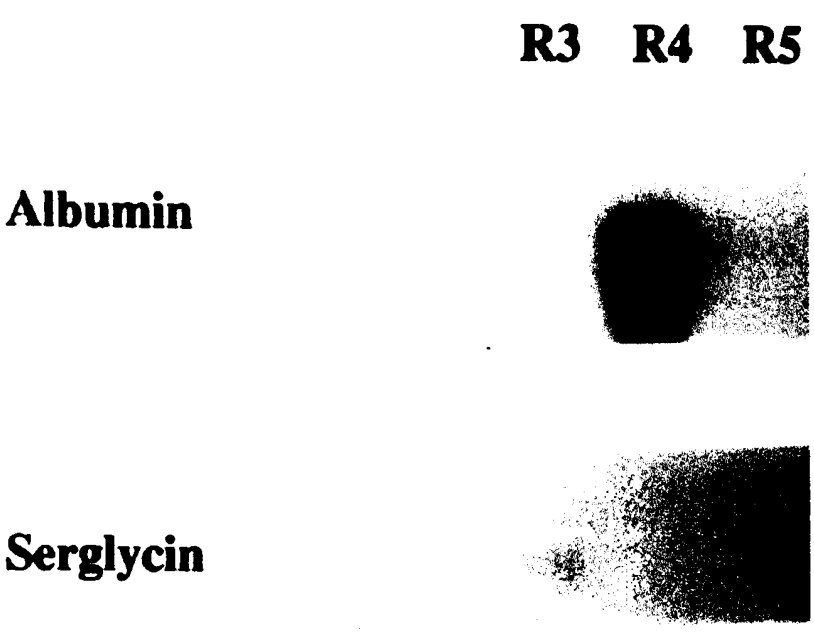


FIG. 3

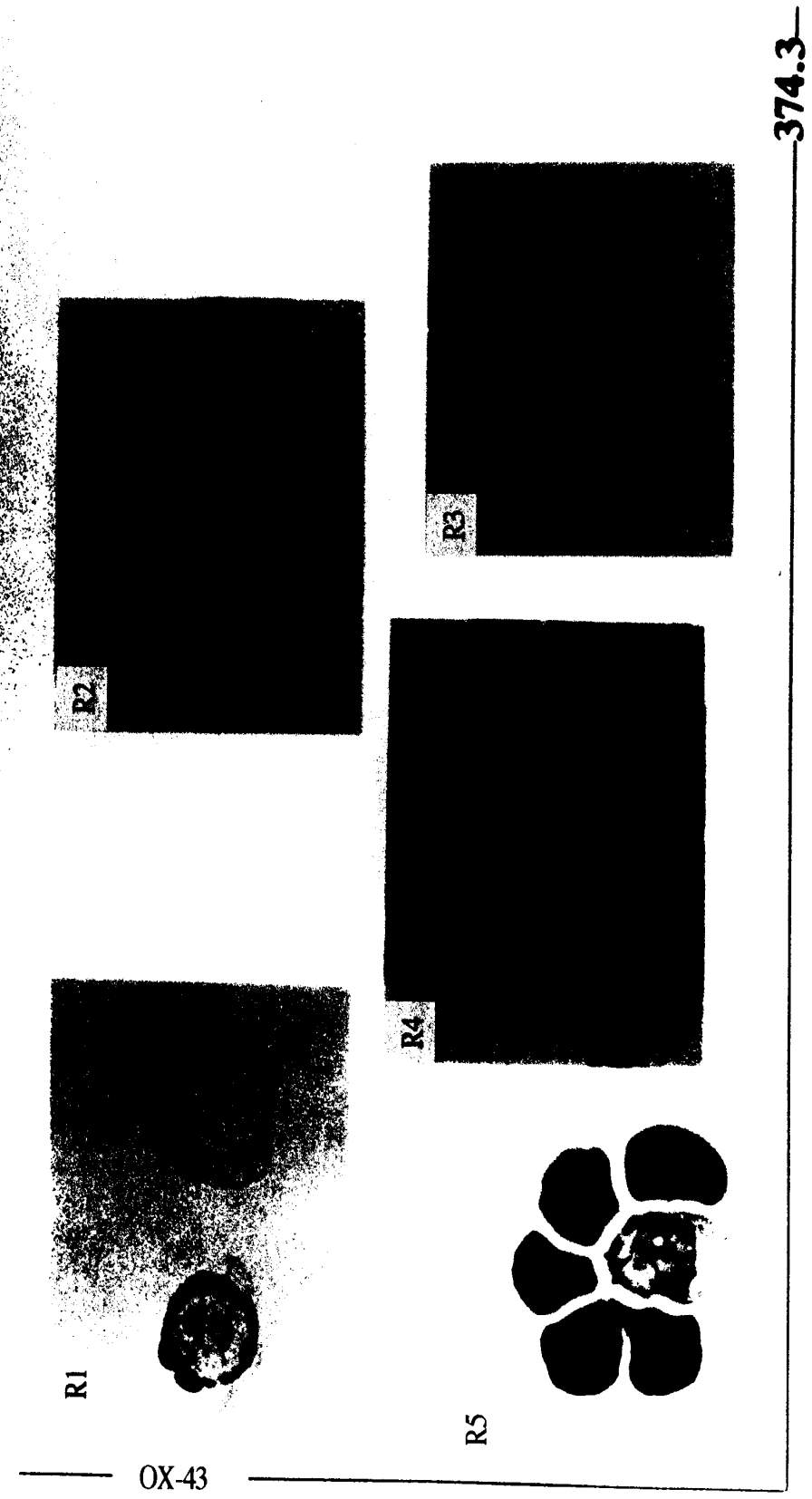


FIG. 4

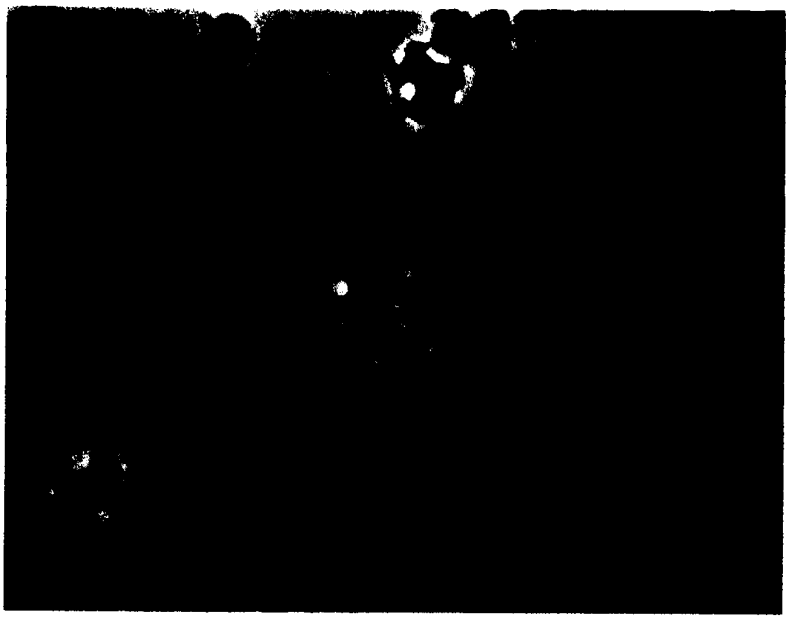


FIG. 7A

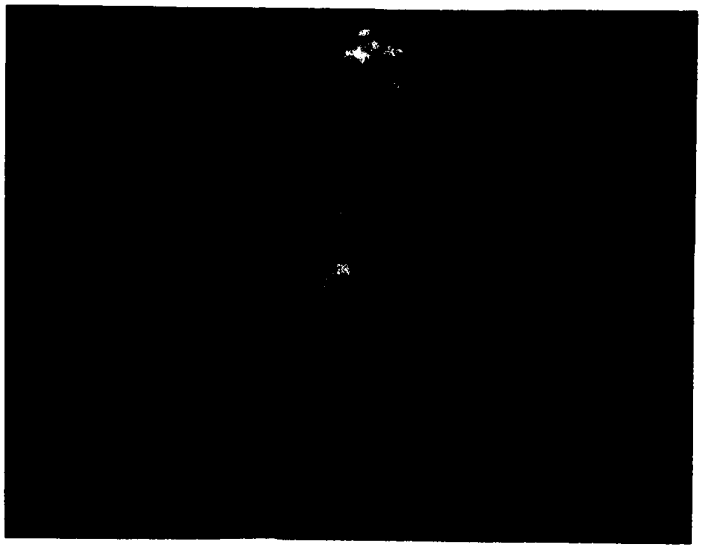


FIG. 7B

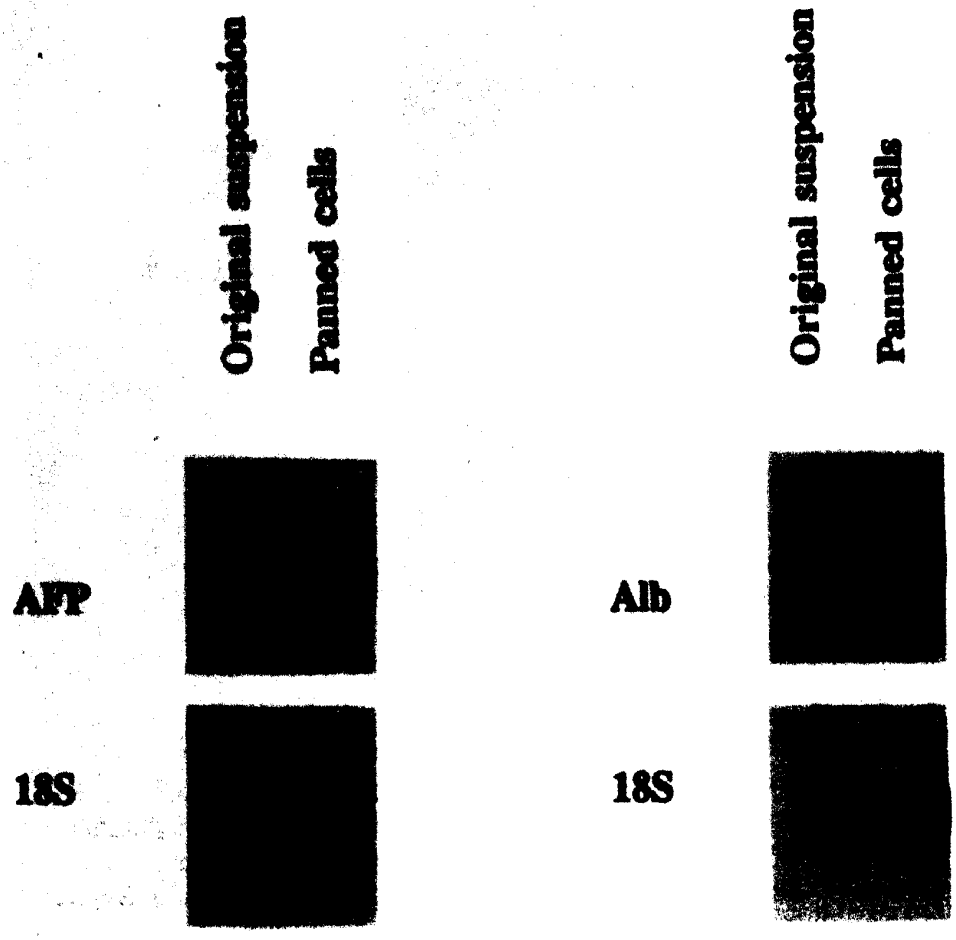

Original suspension
Panned cells
AFP
18S
Original suspension
Panned cells
Alb
18S

FIG. 8

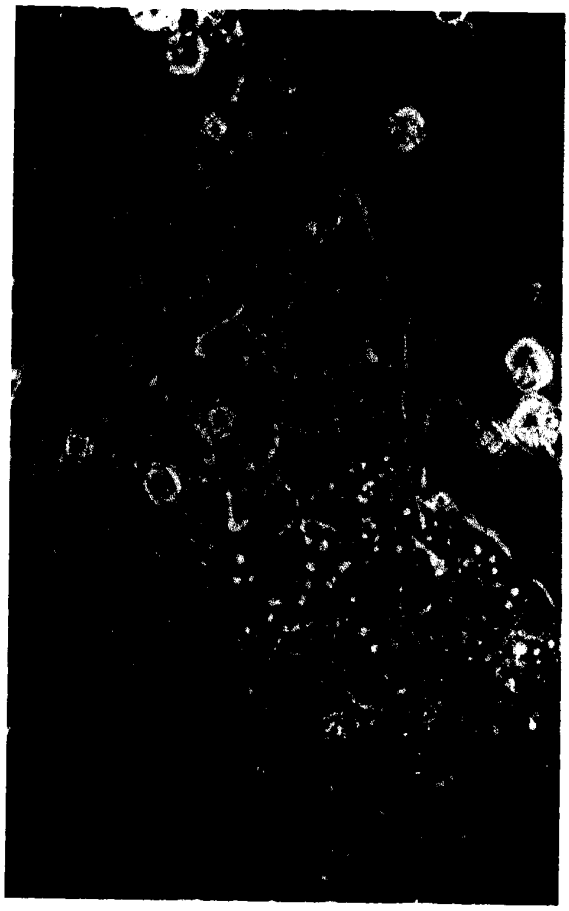


FIG. 10

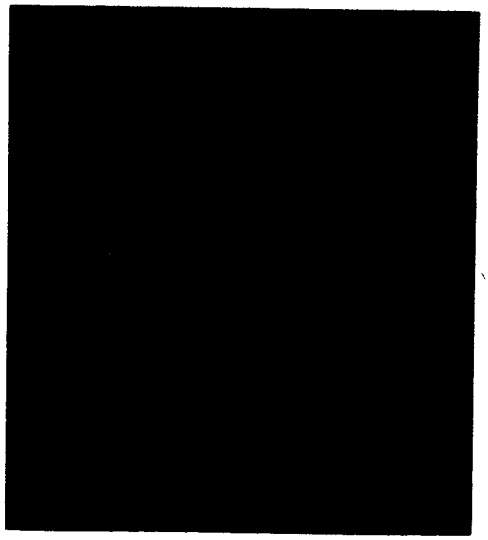


FIG. 11

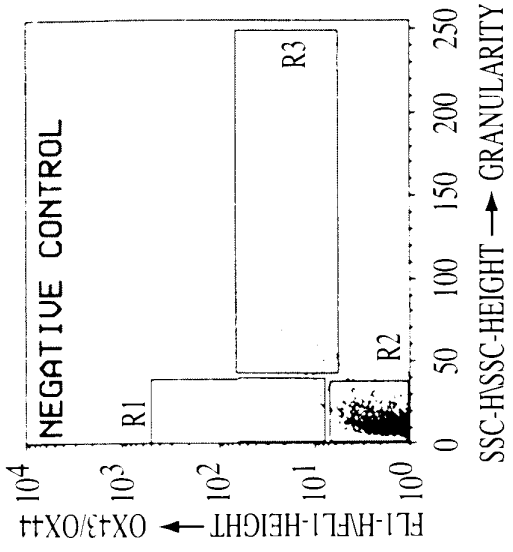


FIG. 9A

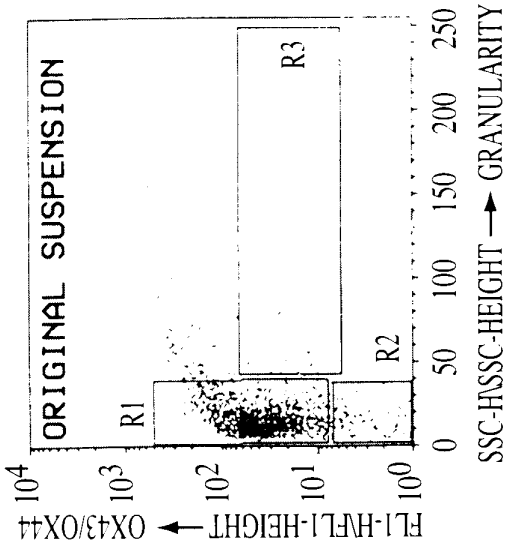


FIG. 9B

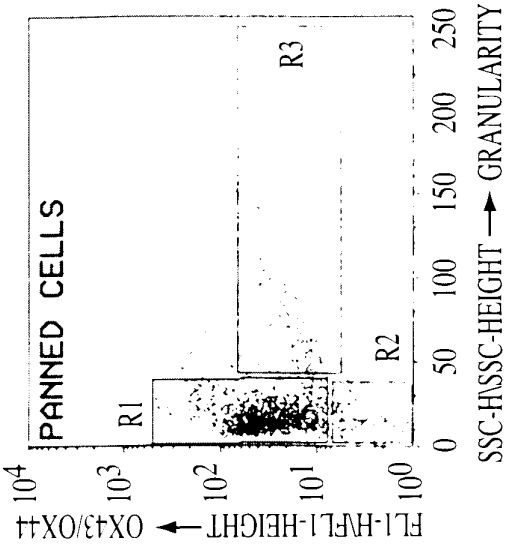


FIG. 9C

FIG. 5B

FIG. 5C

FLOW DIAGRAM OF HEPATOBLAST ENRICHMENT

LIVERS (8-9 mgs)

↓ DIRSPERSION WITH EGTA AND THEN COLLAGENASE

SINGLE CELL SUSPENSION PREPARATION: COLLAGENASE, EGTA, 4° C

↓ 10^7 CELLS/8 mgs LIVER

↓ 3.2 ± 1.3 % ARE ALB^+

↓ 2.5 ± 0.7 % ARE AFP^+

↓ 87.9 ± 2.5 % ARE $OX43/44^+$

PANNING

RED BLOOD CELL PANNING (2X)

↓ 29 ± 5 % OF CELLS REMAIN

↓ 9.5 ± 1.2 % ARE ALB^+

↓ 9.8 ± 0.9 % ARE AFP^+

↓ 80.4 ± 3.9 % ARE $OX43/OX44^+$

OX-43/OX-44 PANNING (MYELOID AND ENDOTHELIAL CELLS)

↓ 16 ± 4 % OF CELLS REMAIN

↓ 14.8 ± 3.6 % ARE ALB^+

↓ 14.9 ± 2.5 % ARE AFP^+

↓ 69 ± 10 % ARE $OX43/OX44^+$

FLUORESCENCE ACTIVATED CELL SORTING

NEGATIVELY SORT FOR CONTAMINANT CELL POPULATIONS:

OX-43(CD)/OX-44(CD37)$^+$ CELLS = PRECURSORS AND MATURE FORMS OF HEMOPOIETIC CELLS (MYELOID, ERYTHROID) AND ENDOTHELIAL CELLS

OF REMAINING CELLS (OX-43$^-$ +OX-44$^-$ CELLS), SORT FOR CELLS VARYING IN OC.3 EXPRESSION AND GRANULARITY:

OX-43(CD)/OX-44(CD37)$^+$ CELLS = MOSTLY HEPATIC PRECURSORS, SOME RESIDUAL HEMOPOEITIC CELL CONTAMINANTS, STROMAL CELLS

OC.3$^-$, GRANULAR CELLS = COMMITTED BILE DUCT PRECURSORS (AFP^+, ALB^-)

OC.3$^-$, GRANULAR CELLS = COMMITTED HEPATOCYTE PRECURSORS (AFP^+, ALB^{+++})

OC.3$^+$, AGRANULAR CELLS = EARLY HEPATOBLASTS (AFP^{+++}, ALBUMIN$^+$ AND CK 19$^-$)

FIG. 6

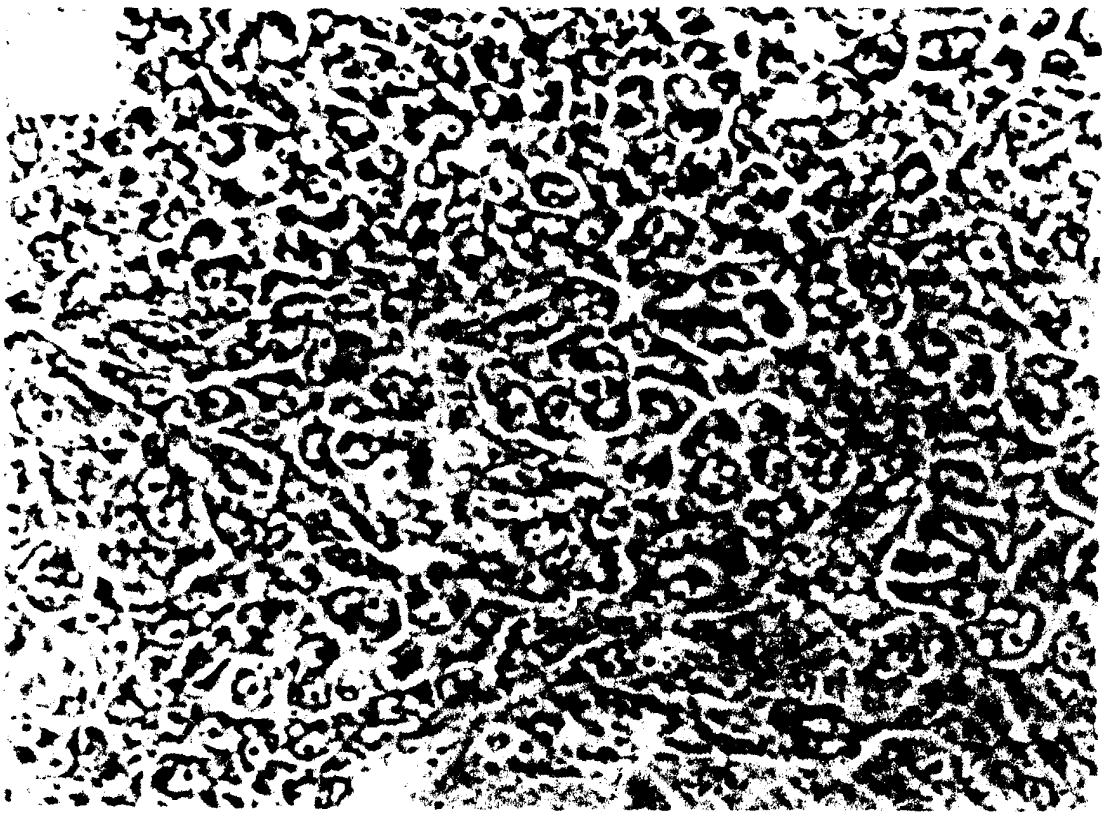


FIG. 5A

Figure 10

Figure 11

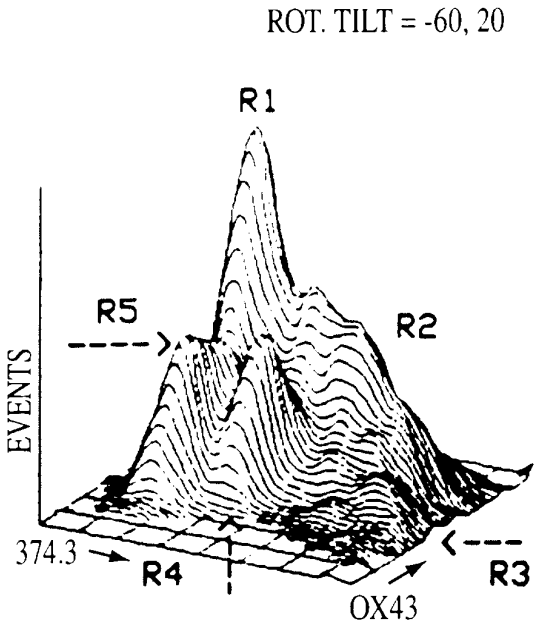


FIG. 1A

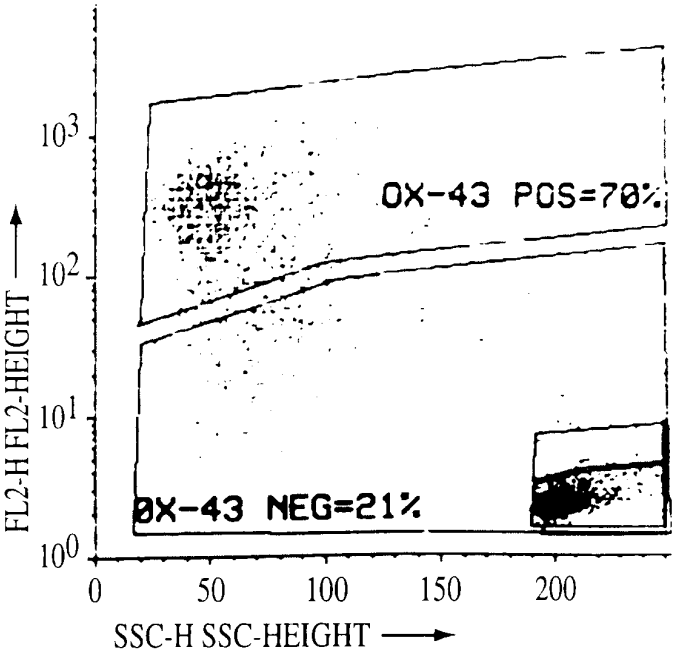


FIG. 1B

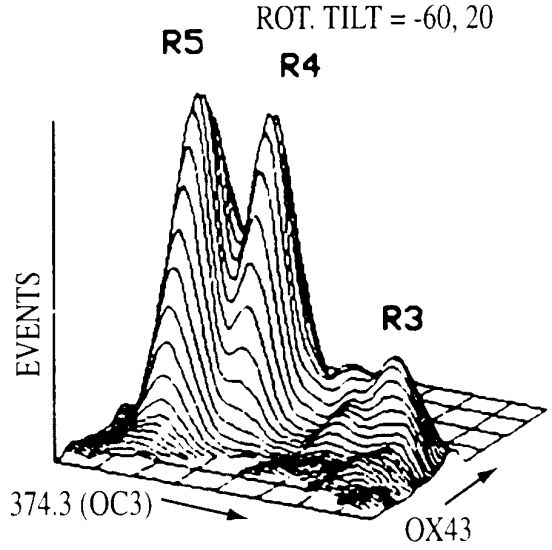


FIG. 1C